IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Clinton et al.
Filing Date: 16 February 2000
Serial No.: 09/506,079
For: HER-2 BINDING ANTAGONISTS
Art Unit: 1642
Examiner: Anne L. Holleran
Docket: 49321-16
Date: 28 January 2005

Mail Stop Amendment
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

AFFIDAVIT OF DR. GAIL M. CLINTON UNDER 37 C.F.R. § 1.132
(IN SUPPORT OF RESPONSE AND AMENDMENT UNDER37 CFR § 1.111)

Sir or Madam:

I, Dr. Gail Clinton, being duly sworn, say:

1. I am an inventor of the subject matter described in the above-identified pending patent application.

2. I am presently employed as an Associate Professor at Oregon Health and Science University in Portland, Oregon (from 1/01/87 to present). I received a Bachelor of Science Degree in 1969 from the University of California, San Diego, and a Ph.D. degree from the University of California, San Diego in 1974. I completed a postdoctoral fellowship at Harvard Medical School in 1981.

3. I am an author or co-author of more than 50 peer-reviewed research articles in the field of oncogene regulation and I am a member of a number of scientific and medical societies, most notably American Association of Cancer Research. I have received a number of prizes and

awards for achievement in research. Most notably, I was the recipient of a postdoctoral fellowship from the American Cancer Society, numerous grants from the National Cancer Institute, and was awarded a Fogarty Senior International Fellowship. I have served on several peer review groups and study sections and have been invited to give numerous presentations on my research at national and international meetings.

4. I have read the above-identified patent application, and the attached Response and Amendment, and it has been explained to me that particular claims have been rejected by the Patent Examiner based on an alleged lack of enablement for binding and biological activity of various Herstatin polymorphisms: variants 1-10 (corresponding to SEQ ID NOS:19-28 and 29-38); and the "most common sequence" (wild-type "wt" sequence) shown in Figure 8 of the present application (corresponding to SEQ ID NOS:14 and 15). I understand that the Examiner is questioning whether, apart from the Herstatin that was previously disclosed and claimed (variant 11), the Herstatin polymorphisms (including the most common sequence) bind to HER-2 and inhibit its activity.

5. This data provided in this affidavit was obtained under my supervision and follows the teachings of the present application and standard methods as described herein. Some of the data has been published subsequent to the present application (*i.e.*, Shamieh L and Clinton GM, *FEBS Lett*, vol 568, pp163-166 (2004) (attached hereto). A comparison of the binding and biological activities among several Herstatin polymorphic forms, two of which (i.e., variant 11 and the "wt" sequence) are described in the present application, shows that Herstatin allelic variants have comparable HER-2 binding and biological activity in inhibiting HER-2 dimerization on cancer cells.

6. *Materials and Methods.*

Cells—HER-2 overexpressing 3T3 cells are known in the art (*e.g.*, Lin and Clinton, Oncogene 6:639-643, 1991) and are the same as those used in the present application.

Herstatin proteins—The alleles studied are wild-type ("wt"), "L/N", and "R to C". Additionally, a rare mutant referred to in Shamieh & Clinton as "R to I" was investigated. Wild-type "wt" corresponds to the most common sequence (*i.e.*, to that of Figure 8 of the present application, corresponding to present SEQ ID NOS:14 and 15). The "L/N" variant corresponds to the previously claimed variant 11 and contains two substitutions (double variant) in the intron 8 encoded ECDIIIa domain; namely Pro to Leu, and Asp to Asn at amino acids positions 6 and 73, respectively, relative to the "wt" form. The "R to C" variant contains a substitution of Cys in place of Arg at amino acid position 17 of the intron encoded region. The "R to I" encoded ECDIIIa allelic variant with a Arg –> Ile substitution at amino acid position 31 of the ECDIIIA region.

Purification of proteins—To obtain polypeptides corresponding to the intron 8 – encoded region of Herstatin, the various polymorphic intron 8 cDNAs were expressed as poly-His proteins using a pET30 bacterial expression vector and BL-21 cells. To obtain full length Herstatin (and variants), the "wt" Herstatin (with an N-terminal poly Histidine tag) in an insect expression vector, was mutagenized to generate the "L/N," "R to C, and R to I" forms.. S2 insect cells were stably transfected with the "wt" and mutant forms, their expression was induced by copper, and each was purified by nickel affinity chromatography.

Binding assays—Binding of Herstatin polymorphic proteins to HER-2 overexpressing 3T3 cells was performed by standard methods as taught by the present application. The binding comparison was done by adding 100 or 500 nM protein to HER-2 overexpressing 3T3 cells or to parental 3T3 cells, incubating the cells on ice for 2 hrs, washing the cells twice with PBS, extracting the cell cultures, and measuring the bound Herstatin by Herstatin-specific ELISA (Upstate) as described in Shamieh L and Clinton GM, *FEBS Lett*, vol 568, pp163-166 (2004) (attached hereto). The results (Figure 1) are plotted as the direct ELISA reading of Herstatin bound to 3T3/HER-2 cells, with the background binding to parental 3T3 cells subtracted.

Inhibition of growth of DU145 (ErbB receptor family over-expressing) prostate cancer cells—On day one, ~40,000 DU145 cells/well in 12-well plates were washed and treated in triplicate with either control vehicle or concentrations from 100 to 300 nM of purified "wt", "R to C,"

"L/N", or "R to I" Herstatin forms, in media with 0.1% fetal bovine serum. On day 3, the cells were treated a second time and on day 5, viable cells were quantified by a standard MTS cell assay (*e.g.*, as described in Romero-Jhabvala et al., *Oncogene* Vol 22, pp 8178-8186 (2003)). The results (Figures 2 and 3) are plotted as % inhibition relative to vehicle-treated control.

Shamieh & Clinton—The Materials and Methods and Results of Shamieh L and Clinton GM, *FEBS Lett*, vol 568, pp163-166 (2004) (attached hereto) are incorporated by reference into this Affidavit, and the methods therein with respect to expression and purification of Herstatin, as well as those pertaining to Herstatin binding, follow the teachings of the present application.

7. *Results.*

Figure 1 (attached hereto) shows a direct comparison of HER-2-specific binding to HER-2 overexpressing 3T3 cells, among the following allelic variants of Herstatin: the most common form ("wt"); the "L/N" variant (the previously claimed variant 11); and an "R to C" ECDIIIa region variant that is the subject of another pending patent application. All three allelic variants of Herstatin show comparable binding at nM concentrations.

Figure 2 and **Figure 3** (attached hereto) show a direct comparison of inhibition of the growth of DU145 prostate cancer cells that over-express the ErbB receptor family (including HER-2, EGFR and HER-3 receptors), among the following variants of Herstatin: "wt" (most common sequence); the "L/N","R to C", and "R to I" polymorphic forms of Herstatin. The results show that all three Herstatin allelic forms had strong, comparable activity in inhibiting the growth of this ErbB receptor family-expressing cancer cell line.

Shamieh L and Clinton (attached hereto) show binding interactions (using HER-2 overexpressing 3T3 cells and HER-2 transfected Cos-7 cells) of Herstatin and the intron-encoded domain thereof with receptors of the ErbB receptor family including HER-1 (EGFR), HER-2, HER-3, HER-4, and additionally to IGF-1R. Both "wt" Herstatin (corresponding to present SEQ ID NO:15) and the corresponding intron-encoded portion thereof (corresponding to present SEQ

ID NO:14) bind at nM concentrations to HER-2 and to EGFR (and also to ΔEGF-R, HER-4 and IGF-1R). While the initial published results indicate that an "R to I" encoded ECDIIIa allelic variant (Arg –> Ile at amino acid position 31 of the ECDIIIA region, and the subject of another patent application) may eliminate binding of the corresponding intron-encoded portion to HER-2. Our current data (Figure 3) shows, in terms of percent inhibition versus concentration, that the corresponding full-length Herstatin, comprising this variant has dose responsive (100 and 300 nM were tested) bioactivity against ErbB family receptor overexpressing DU145 prostate cancer cells that is comparable to that of the most common "wt" allelic variant. This indicates that in the full length Herstatin protein, the R to I variation does not block receptor interaction required for bioactivity.

8. In conclusion, the data described herein, including that described in Shamieh L and Clinton GM, *FEBS Lett*, vol 568, pp163-166 (2004), which was obtained by following the teachings of the present application, shows that Herstatin allelic variants, including the most common one (corresponding to SEQ ID NOS:14 and 15), have strong HER-2 binding and activity in inhibiting growth in HER-2 expressing cancer cells that is comparable to the previously claimed polymorphic variant 11. Additionally, the allelic variants have comparable binding and biological activity in EGF expressing cancer cells.

9. I further declare that all statements made herein of my own knowledge are true and that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Gail M. Clinton

State of)
) ss.:
County of)

On this 28th day of January, 2005, before me, a Notary Public in and for the State and County aforesaid, personally appeared Gail M. Clinton, to me known and known to me to be the person of that name, who signed and sealed the foregoing instrument, and she acknowledged the same to be her free act and deed.

Notary Public

Commission expires 10/28/08

OFFICIAL SEAL
J. PELLING
NOTARY PUBLIC-OREGON
COMMISSION NO. 386324
MY COMMISSION EXPIRES OCTOBER 28, 2008